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SHORT COMMUNICATION

Relationship between litter characteristics and female size in *Tityus stigmurus* (Scorpiones, Buthidae)

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Abstract. *Tityus stigmurus* (Thorell 1876) is one of the most medically important scorpion species in Brazil, but many basic aspects of its life history are unknown. Here the pattern of female reproductive investment was examined, along with development of the 1st and 2nd instars and the relationship between 2nd instar mass and molting to the 3rd instar. Relative to other buthid scorpions, *T. stigmurus* has a smaller litter (average 10 young) and a shorter 1st-instar period (average 4 days) and 2nd-instar period (average 68 days). Neither litter size nor offspring mass showed a relationship to female size. A significant positive correlation was observed between total litter mass and litter size. The minimum mass required for successful molting to the 3rd instar was 34.0 mg. Overall, female reproductive resources in *T. stigmurus* appear to be applied to the production of more but not heavier offspring.

Keywords: Litter size, female size, reproduetive investment, offspring mass, intermolt period

Differences in female reproductive investment are common in closely related species of animals. A female may use her finite reproductive resources to maximize her fitness by producing either large litters of small young or small litters of large young. Given that the individual fitness of each offspring is generally correlated with larger maternal investment, the female's option of producing large litters with small young creates a potential conflict in the fitness of the female and her young. Thus, the optimal distribution of parental resources tends to be based on the cost-benefit relation for both parents and offspring (Smith & Fretwell 1974; Stearns 1992; Fox & Czesak 2000).

In scorpions, studies on the female reproductive investment are still scarce, yet such studies have shown that different taxa show different tendencies. In species such as *Centruroides exilicauda* (Wood 1863), *Vaejovis spinigerus* (Wood 1863), *Diplocentrus peloncillensis* Francke 1975, and *Pseudouroctonus apacheanus* (Gertsch & Soleglad 1972) there is no relationship between female size and offspring production (Brown 2004). However, a higher reproductive investment in larger litters was recorded in the largest females in *Centruroides vittatus* (Say 1821), *Vaejovis spinigerus* (Wood 1863), and *Tityus columbianus* (Thorell 1876) (Formanowicz & Schaffer 1993; Lourenço et al. 1996; Brown 2004). Although *Tityus* species are capable of producing multiple litters from a single insemination (Kovoor et al. 1987; Polis & Sissom 1990; Lourenço et al. 1996), little is known about female reproductive investment, including species involved in medically important envenomations in Brazil.

Population dynamics are also influenced by the duration of postembryonic development. Within Buthidae, this varies from six to 48 months depending on the species (Polis & Sissom 1990). Both female reproductive investment and postembryonic duration are unknown for *Tityus stigmurus* (Thorell 1876), a species that is widely distributed in urban areas of northeastern Brazil and responsible for many reported envenomations each year (Eickstedt 1983, 1984; Lira-da-Silva et al. 2000; Barbosa et al. 2003). Its occurrence in urban settings reflects its ability to live under roofs, among accumulated debris in the exterior areas of residences (Eickstedt 1983, 1984; Lourenço et al. 1996), and in cesspits.

This study analyzed the litter size, offspring mass, and total litter mass of *T. stigmurus* and evaluated the relation of female size to these variables. Times of development for 1st and 2nd instars, as well as the relationship between mass and molting to the 3rd developmental instar, were also assessed.

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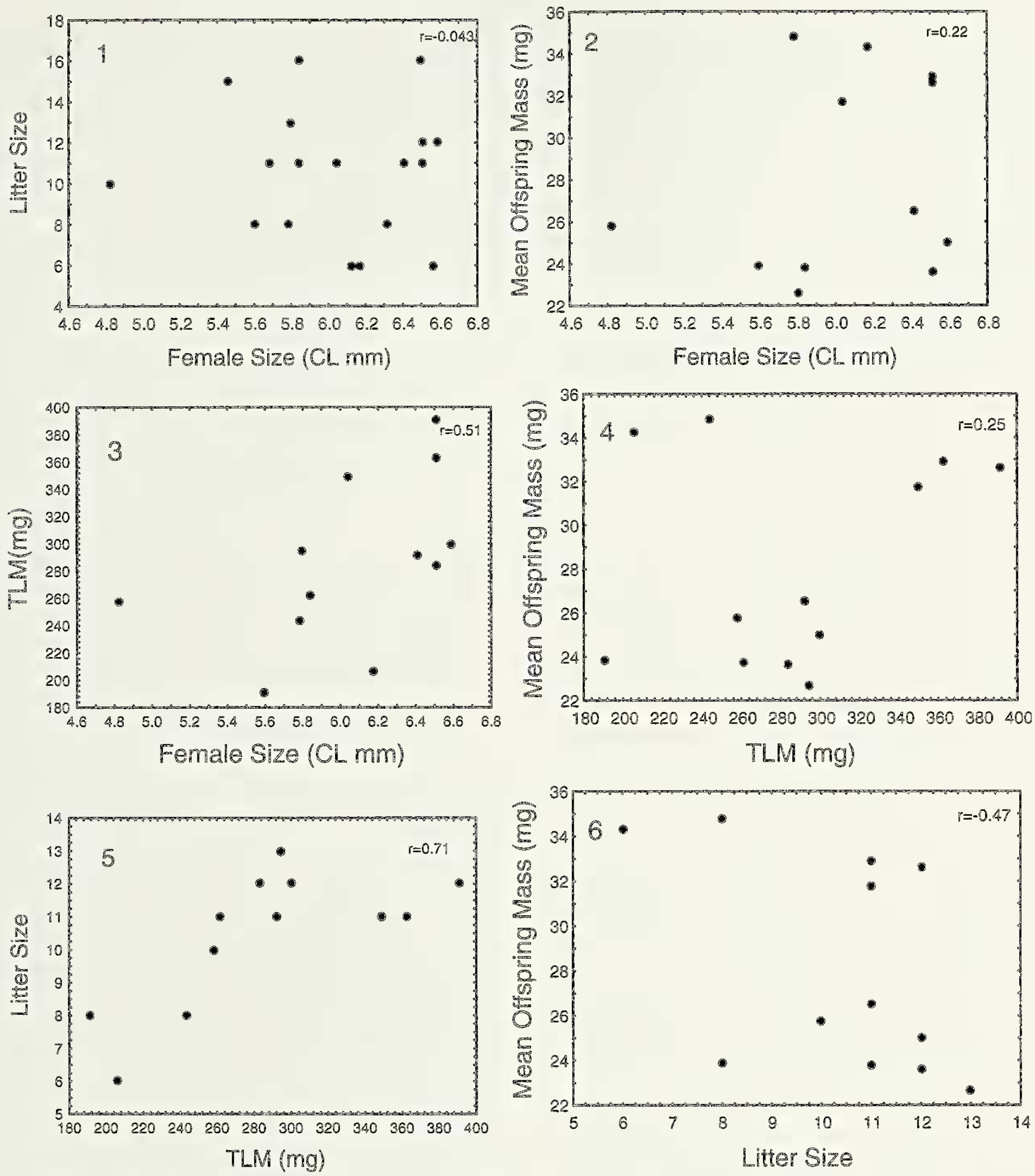
METHODS

Animal sampling and maintenance.—*Tityus stigmurus* adults were collected from residential areas of Recife (8°04′03″S, 34°55′00″W), Pernambuco State. The climate is predominantly hot and wet with mean temperature 25° ± 2° C and annual pluviometric precipitation of 2.094 mm. Following capture, animals were individually housed in plastic terraria (8.5 cm diameter × 7.8 cm height) where water and shelter were made available. Weekly, *Periplaneta americana* (Linnaeus 1758) nymphs were offered as food. The animals were kept at 28° ± 3°C mean temperature and 12:12 h light/dark photoperiod.

Data collection and analysis.—The relationship between maternal carapace length (CL) and litter size (LT) was analyzed in a sample of 25 pregnant females that gave birth to young. In 14 broods, it was possible to analyze 1st instar duration and to determine total litter mass (TLM) and mean offspring mass (MOM). TLM was taken as a measure of reproductive investment and was calculated as the sum of individual masses of juveniles according to the methodology of Brown (2004). Juveniles were weighed to the nearest 0.1 mg with an analytical balance (Mettler AE 260 DeltaRange) as soon as they moved down from the mother's dorsum. After offspring dispersal, female CL was measured with digital calipers to the nearest 0.1 mm under a dissecting microscope (Leica MZ6).

Litter size was estimated by counting juveniles on the female's dorsum within 24 h of birth and again immediately after dispersal. All dead and living juveniles were counted except for those cannibalized. Due to a female's habit of cannibalizing young at birth and the superimposed distribution of young on her dorsum, an aceurate litter size was difficult to obtain. Eight gravid females were dissected and the number of young recorded for litter size comparison. Females chosen for dissection showed signs of imminent parturition such as lack of movement and an enlarged mesosoma with offspring visible through the integument. In general, females presenting these traits gave birth within two weeks.

ANOVA was used to test the differences between brood sizes. The correlation coefficient (Pearson's *r*) was used to evaluate the relationships between female CL and litter size, mean offspring mass or TLM. It was also used to examine the relationship between TLM and litter size or MOM. The significance of these correlations was assessed with a t-test.



Figures 1–6.—Correlation results between carapace length (CL) and brood characteristics of *Tityus stigmurus*. 1. Litter size (LT); 2. Mean offspring mass (MOM) 3. Total litter mass (TLM); 4. Correlation between total litter mass and mean offspring mass. 5, 6. Correlation between total litter mass (TLM) against litter size and litter size, respectively, with mean offspring mass in *Tityus stigmurus*.

Duration of first and second instars and mass variation in the latter.—The duration of the 1st instar stage was determined for 25 litters by counting the days between birth and the first molt. A second sample of 19 newly dispersed individuals was used to determine the period between the 1st and 2nd molts. Between molts, the animals were fed weekly with nymphs of *P. americana* with mean mass of 7.8 mg. Masses of 2nd instars were determined 24 h after each feeding in order to estimate variation in mass and to determine any relationship between mass and molting. The difference between the initial mass (Mi), recorded up to 24 h after dispersal, and the final mass (Mf), recorded immediately prior to the 2nd molt, was used as an estimate for the mass variation in the 2nd instar. The lowest Mf was used as an estimate of the minimum mass needed to molt successfully.

RESULTS

Tityus stigmurus showed great variation in female size, litter size, and mean offspring mass. Female size (CL) averaged 6.00 ± 0.44 mm, varying from 4.82 to 6.59 mm. The litter size (LS, n = 25 females) averaged 10.40 ± 2.90 and was unrelated to female size (Fig. 1). Similar data were obtained in the group used to analyze total litter mass (TLM) and mean offspring mass (MOM) (n = 14 females) (CL = 6.03 ± 0.48 mm and LS = 10.14 ± 2.11, respectively). MOM in this group averaged 27.67 ± 4.8 mg and was not related to female size (Fig. 2). The mean brood size obtained from females dissected before parturition (n = 8; 11.87 ± 4.09; range 8–21) was not significantly different from those that gave birth to live young ($F_{(0.05;1;23)} = 0.62$; $P = 0.55$).

The reproductive investment (TLM) showed a positive, but not significant, correlation with female size (Fig. 3) and mean offspring mass (Fig. 4). However, a significant positive correlation was found between TLM and litter size (Fig. 5). A negative but not significant correlation was found between litter size and mean offspring mass (Fig. 6).

Female mass before parturition (until after the last feeding) ranged from 975.4 to 1655.0 mg, showing a TLM-to-female-mass ratio higher than 0.20 for most females (n = 9/12, ranging from 0.13 to 0.42).

Table 1.—Data on reproductive investment and first instar traits of 12 broods of *Tityus stigmurus*. CL = maternal carapace length.

CL (mm)	Litter size	1st instar Period (days)	Mean offspring mass (mg)	Total litter mass (mg)
6.41	11	4	26.5 ± 1.7	292.0
5.84	11	5	23.8 ± 3.2	261.4
6.17	6	4	34.3 ± 3.0	205.9
6.51	12	4	23.6 ± 1.0	283.7
4.82	10	3	25.8 ± 2.6	257.8
6.51	11	5	32.9 ± 4.1	362.3
6.51	12	5	32.6 ± 3.0	391.5
6.04	11	3	31.7 ± 3.1	349.3
5.60	8	4	23.9 ± 2.1	190.9
5.78	8	4	34.1 ± 3.0	272.6
6.59	12	3	25.0 ± 0.5	299.7
5.80	13	7	22.7 ± 1.6	294.4
6.02	10	3	29.9 ± 2.73	298.8
5.81	7	3	24.1 ± 3.08	168.6

Birth, first instar and pre-dispersal phase.—The duration of the first instar was investigated in a sample of 142 individuals from different litters. This period was on average 4.07 ± 1.14 days (range 3–7) (Table 1) and the molt occurred simultaneously in offspring of the same litter. After the first molt the immature individuals remained on the mothers dorsum for 5–6 more days. Dispersal occurred 5–10 days following birth. Individual young occasionally climbed down from the female's dorsum, particularly in the nocturnal period, then returned to the mother. While off the female, they searched for water and food in the environment. After the transition period from dorsum to the environment, they remained apart from the mother, though aggregated in the shelters placed in the terraria.

Second instar phase.—The intermolt period between the 2nd and 3rd instar (n = 19) was approximately 16 times longer than the duration of the 1st instar. The simultaneity observed in the first molt did not occur in the second, and the duration of this phase of development was on average 67.81 ± 18.80 days (ranging = 42–107 days). The mortality for the 2nd instar was 47.37%, and among these 33.33% died during the intermolt period (one due to cannibalism) and 66.67% died during the molting process (Table 2). No relationship was observed between mass of the offspring before a molt and survival during the molt. The minimal pre-molt mass of a surviving individual was 34.0 mg and the maximum was 59.0 mg. However, many immatures with masses ranging between 49.0 mg and 56.0 mg died during or after the molt.

DISCUSSION

Tityus stigmurus can vary greatly in female size, litter size, mean offspring size, and total litter mass, as described for other scorpion species both within and among populations (Brown 2001, 2003). These traits are important components of female reproductive investment, and impact the fitness of both the female and her offspring.

Based on data summarized by Polis & Sissom (1990) and Lourenço (2007), *T. stigmurus* had lower brood sizes (mean of 10 young) compared to most buthid species (average 32) and other species of *Tityus* (mean variation = 15–25). Our results show that females that alloeated more resources to reproduction (measured as TLM) had more but not heavier offspring, and that TLM was not significantly related to female size (CL). This may explain the lack of correlation between CL and litter size. There are few studies on the reproductive investment in *Tityus* species, although Lourenço et al. (1996) found a positive correlation between litter size and female size in both parthenogenetic and sexual populations of *T. columbianus*. It is important to note that our study used females that had been maintained in the laboratory for at least a year and that data such as prey availability and specimen age were not controlled. Thus, variation in prey availability in the environment may have led to differential resource allocation by females resulting in large variation in TLM. A shift in offspring size from negative to positive values in *V. vorhiesi* Stahnke 1940 was attributed to an increase in prey availability in the environment, which allowed females to invest more in reproduction (Brown 2001). In addition, old females tend to reduce the number of follicles (Lourenço 1979) which may lead to small litter sizes. Therefore, lack of correlation between most of our variables may have been influenced by factors such as accessibility of food in the environment and the age of females.

In contrast, in several speeies such as *Centruroides exilicauda*, *Vaejovis spinigerus*, *Diplocentrus peloncillensis*, *Pseudouroctonus apacheanus* (Brown 2004) and *Centruroides vittatus* (Formanowicz & Shaffer 1993), larger females invest more in reproduction. In these species, a positive correlation between female size and total litter mass has been described. Moreover, litter size was often positively related with CL and TLM, showing that the largest females invest more in reproduction through production of more offspring. In all of these speeies, as well as in *T. stigmurus*, there was no correlation between female size and mean offspring mass, indicating that larger females do not produce larger offspring. Offspring mass in *T. stigmurus* was also not correlated with litter size, which may suggest a lack of trade-off between mass and number of individuals. Similar results were obtained by Salomon et al. (2005) in the spider *Stegodyphus lineatus* Latreille 1817. Offspring mass in this spider showed no correlation with clutch size, indicating that each variable may be determined independently. In addition, offspring mass at hatching (and consequently egg size) appeared to be relatively constant and independent of female size and body mass.

In all of the observed litters, juveniles remained unfed on the mother's dorsum during the entire 1st instar phase and underwent the 1st ecdysis simultaneously, a phenomenon characteristic of many scorpion species (Sissom & Francke 1983; Polis & Sissom 1990; Brown 1997, 2004; Lourenço 2000; Farley 2005; Lourenço & Goodman 2006). Mean first-instar duration in *T. stigmurus* (4 days) was shorter than other Buthidae, including *Centruroides gracilis*

Table 2.—Development length (days) and mortality rates in *Tityus stigmurus* 2nd instar.

Litter size	Second instar period, mean ± SD (range)	Mortality (%)
7	72.2 ± 22.70 (52–107)	85.71
2	64.5 ± 20.51 (50–79)	0
1	56	0
8	69.12 ± 17.00 (50–95)	25.00
1	42	100
19	67.81 ± 18.80	47.37

(Latreille 1804) at 8 days (Francke & Jones 1982), *Centruroides exilicauda* (Wood 1863) (= *C. sculpturatus*) at 7 days (Brown 2004), and *Grosphus hirtus* Kraepelin 1901 at 14 days (Lourenço & Goodman 2006). The first-instar period in *T. stigmurus* is even shorter than that of congeneric species (Polis & Sisson 1990).

Similarly, the 2nd instar of *T. stigmurus* lasted 67 days on average, which was shorter than in other species of Buthidae. Toscano-Gadea (2004) confirmed 321 days for *T. trivittatus*, and Lourenço & Goodman (2006) described a 112 day period for *G. hirtus*. A longer developmental period for *T. stigmurus* was described by Matthiesen (1971), who found an average of 80 and 148 days in two broods of five young each produced by a single female. Scorpions from both studies are likely to have come from different populations since they were obtained from cities separated by 209 km. According to Brown & Formanowicz (1995), individuals from different populations may face an adaptation to microvariation in the environment, and this fact may reflect genetic differences that might explain the differences relative to our findings.

Mortality in the period between the 2nd and 3rd stages was high, as only 52.63% of the initial sample survived. Most of these deaths, 66.67%, occurred during ecdysis, and the body mass at the end of the 2nd stage was not associated with the survival of juveniles. In their experiments with *Vaejovis bilineatus* Pocock 1898, Sissom & Francke (1983) noted that only 43% of the initial sample survived the 2nd molt and suggested dehydration as a possible cause. *Tityus stigmurus* young also appeared to be desiccated after dying during molting, with the integument being dried and frail.

In total, the data gathered in this study suggest that female *T. stigmurus* make a significant investment in reproduction (over 20% of the body mass). It is important to note that the allocation is underestimated given that the neonate scorpions decrease in mass during the first instar period and their first molt (Polis & Sissom 1990). With larger investments in reproduction, there is a trend toward increasing the number but not mass of offspring. Moreover, this trait is not influenced by the mothers size, so that faetors such as age of the female and food availability in the environment are likely to have fundamental importance in the reproductive investment made by this scorpion species. The comparatively short developmental period of this species may promote rapid populational growth when environmental conditions are favorable. Considering that this analysis is restricted to observations of the 1st and 2nd instars, studies of other developmental stages should be conducted to test this hypothesis.

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